# Hepatoprotective effects of systemic ER activation
## TEAD & ER expression in the liver

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```r
# source and library import
source('code/00_helper_functions.R')
library(tidyverse)
library(Seurat)

# color palettes
colPals <- list()
colPals$conditions <- setNames(c('#44AA99', '#117733', '#88CCEE', '#332288', '#DDCC77', '#CC6677', '#AA4499', '#882255'),
                               c('CDf', 'HFDf', 'CDm', 'HFDm', 'DPN', 'DIP', 'E2', 'PPT'))
colPals$RdBu <- rev(RColorBrewer::brewer.pal(n=11, name = 'RdBu'))
colPals$UpDown <- setNames(colPals$RdBu[c(10,2)],
                           c('up', 'down'))
colPals$celltypes <- setNames(c('#B4272F', '#E5462D', '#FFD1D1', '#F4E54C', '#FBAA3E', '#AA654E', '#B58B80', '#6D7AA5',
                                '#B3177E', '#CAC1DD','#67227D','#36B449', '#82C349', '#A9D265', '#199478', '#95A3A3', '#C4C5C7'),
                              c('Cholangiocytes', 'Endothelial cells', 'HsPCs', 'Stromal cells', 'Hepatocytes',
                                'Kupffer cells', 'Monocytes & Monocyte-derived cells', 'T cells', 'NK cells',
                                'ILC1s', 'B cells', 'cDC1s', 'cDC2s', 'Mig. cDCs', 'pDCs', 'Basophils', 'Neutrophils'))
```

## Load data

```r
# mouse bulk RNAseq data
mouse_RNAseq <- list()
# raw counts RNAseq
mouse_RNAseq$raw_counts <- read.table(
  file = 'data/bulkRNAseq_mmus_rawcounts.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE) %>%
  tibble::column_to_rownames('geneID') %>%
  dplyr::select(-PPT_HFD_male_4)
# gene lengths
mouse_RNAseq$gene_len <- read.table(
  file = 'data/bulkRNAseq_mmus_gene_lengths.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE)
# design RNAseq
mouse_RNAseq$design_meta <- read.table(
  file = 'data/bulkRNAseq_mmus_design.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE) %>%
  dplyr::filter(sample != 'PPT_HFD_male_4')
# ensembl gene annotation (Mus musculus)
mouse_RNAseq$gene_ann <- read.table(
  file = 'data/ensembl_mmus_sep2019_annotation.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE,
  fill = FALSE,
  quote = '') %>%
  dplyr::filter(ensembl_gene_id %in% rownames(mouse_RNAseq$raw_counts)) %>%
  dplyr::arrange(factor(ensembl_gene_id, levels = rownames(mouse_RNAseq$raw_counts))) %>%
  dplyr::rename(geneID = ensembl_gene_id) %>%
  dplyr::left_join(mouse_RNAseq$gene_len, by = 'geneID')
# TPM-normalized counts
mouse_RNAseq$tpm <- mouse_RNAseq$raw_counts %>%
  normalizeData(len = mouse_RNAseq$gene_ann$length, method = 'TPM')
```

```
# human gene annotation
human_gene_ann <- read.table(
  file = 'data/ensembl_hsap_dec2021_annotation.tsv',
  sep = '\t',
  header = TRUE,
  quote = '')

# NAFLD patient cohort
cohort_data <- readRDS("data/bulkRNAseq_human_cohort_data.rds")

cohort_data$Govaere$cpm_filt <- cohort_data$Govaere$cpm %>%
  tibble::rownames_to_column(var = 'gene') %>%
  dplyr::filter(gene %in% human_gene_ann$ensembl_gene_id) %>%
  dplyr::mutate(gene = dplyr::recode(gene, !!!setNames(human_gene_ann$external_gene_name,
                                                       human_gene_ann$ensembl_gene_id))) %>%
  dplyr::filter(!duplicated(gene) & gene != "") %>%
  tibble::column_to_rownames(var = 'gene')

# Liver Cell Atlas data from Guilliams et al. 2022 (https://www.livercellatlas.org/download.php)
liver <- readRDS(file = 'data/livercellatlas_feb2022.rds')

# Liver Cell Atlas sample metadata
liver_meta <- read.table(
  file = 'data/livercellatlas_feb2022_sample_annotation.tsv',
  stringsAsFactors = FALSE,
  sep = '\t',
  header = TRUE)

# PHH spheroid RNAseq
PHH_RNAseq_tpm_mean <- read.table(file = 'results/spheroid_TPM_norm_counts_mean.txt',
                                  header = T,
                                  sep = '\t',
                                  quote = '')
```

# TEAD family expression

## Mouse (CD, HFD & HFD + ER treatments)

```
TEAD_genes <- c('Tead1','Tead2','Tead3','Tead4')

df <- mouse_RNAseq$tpm %>%
  tibble::rownames_to_column(var = 'gene') %>%
  dplyr::mutate(gene = dplyr::recode(gene,
                                     !!!setNames(mouse_RNAseq$gene_ann$external_gene_name,
                                                 mouse_RNAseq$gene_ann$geneID))) %>%
  dplyr::filter(gene %in% TEAD_genes) %>%
  dplyr::mutate(gene = factor(gene, levels = TEAD_genes)) %>%
  dplyr::arrange(gene) %>%
  tidyr::pivot_longer(CD_female_1:PPT_HFD_male_3, names_to = 'sample', values_to = 'tpm_expr') %>%
  dplyr::left_join(mouse_RNAseq$design_meta, by = 'sample') %>%
  dplyr::filter(!condition %in% c('CDf','HFDf')) %>%
  dplyr::mutate(condition = factor(condition, levels = names(colPals$conditions)[-c(1,2)]))

df2 <- df %>%
  dplyr::group_by(gene, condition) %>%
  dplyr::select(gene, condition, tpm_expr) %>%
  dplyr::summarize_each(dplyr::funs(mean, sd, se=sd(.)/sqrt(n())), tpm_expr) %>%
  dplyr::arrange(gene, condition)

TEAD_expr_mouse <- df2 %>%
  dplyr::select(gene, condition, mean) %>%
  tidyr::pivot_wider(names_from = 'condition', values_from = 'mean')

p <- lapply(TEAD_genes, function(x){
  ggplot() +
  geom_errorbar(data=df2 %>% dplyr::filter(gene == x), aes(x=condition, ymin=mean-sd, ymax=mean+sd),
                position= position_dodge(width=0.9), width=0.3) +
  geom_col(data=df2 %>% dplyr::filter(gene == x), aes(x=condition, y=mean, fill=condition),
           position = position_dodge(width=0.9), color="black") +
  geom_point(data=df %>% dplyr::filter(gene == x), aes(x=condition, y=tpm_expr),
             shape=21, size=2.5, alpha=0.5, fill="darkgrey", position = position_dodge(width=0.9)) +
  scale_fill_manual(values = alpha(colPals$conditions, 0.9)) +
  xlab("") +
  ylab(paste(x,"expression (TPM)")) +
  theme_bw() +
  theme(axis.text.x =element_text(angle=90, hjust=0.95, vjust=0.5),
```
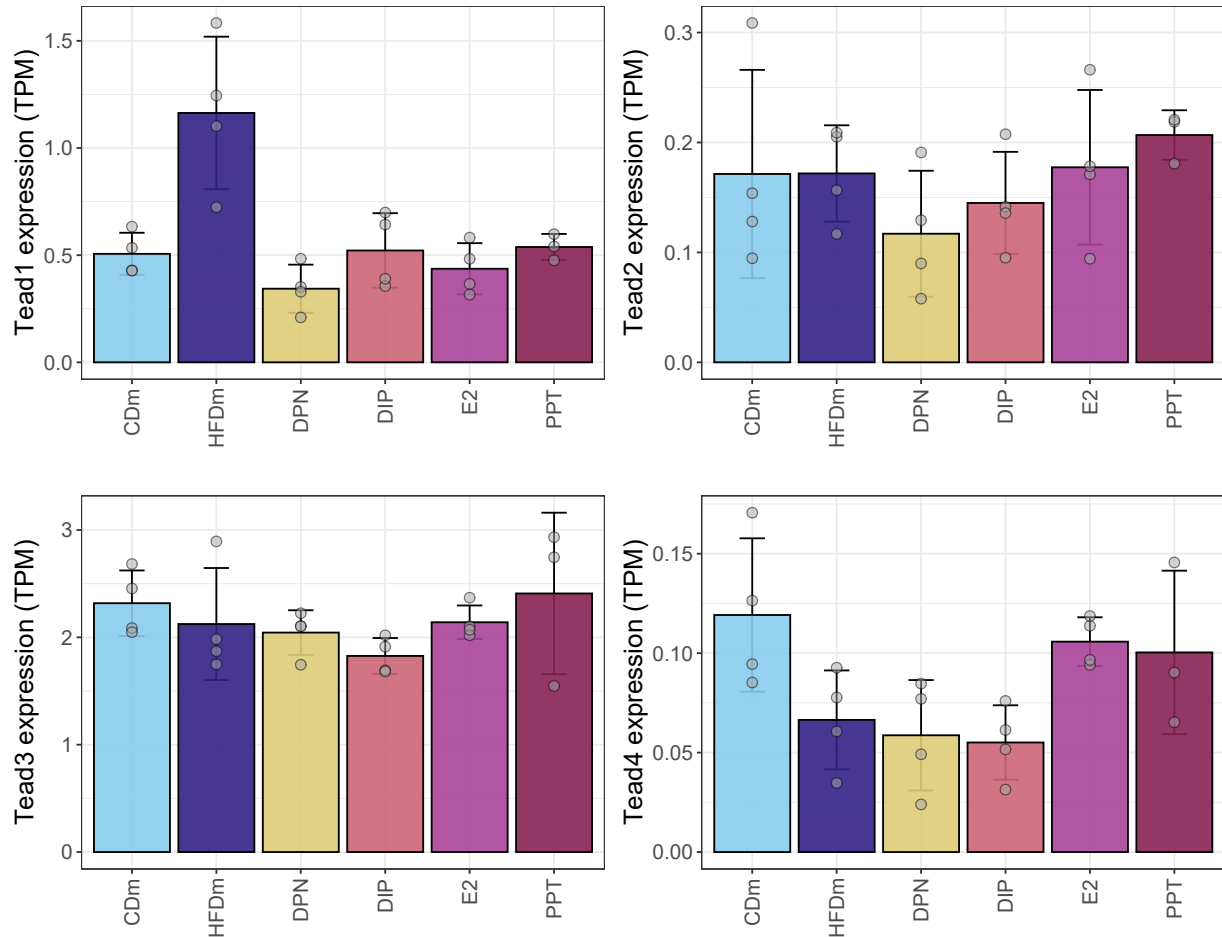
```
        text = element_text(size=15)) +
  theme(legend.position="none")
})

patchwork::wrap_plots(p, nrow=2, ncol=2, byrow=T)
```



## Human NAFLD patient cohort

```
TEAD_genes <- c('TEAD1','TEAD2','TEAD3','TEAD4')

df <- cohort_data$Govaere$cpm_filt %>%
  tibble::rownames_to_column(var = 'gene') %>%
  dplyr::filter(gene %in% TEAD_genes) %>%
  dplyr::mutate(gene = factor(gene, levels = TEAD_genes)) %>%
  dplyr::arrange(gene) %>%
  tidyr::pivot_longer(cohort_data$Govaere$meta$Patient, names_to = 'Patient', values_to = 'cpm_expr') %>%
  dplyr::left_join(cohort_data$Govaere$meta, by = 'Patient') %>%
  dplyr::mutate(Stage = factor(Stage, levels = c('CTRL','NAFL','NASH')))

df2 <- df %>%
  dplyr::group_by(gene, Stage) %>%
  dplyr::select(gene, Stage, cpm_expr) %>%
  dplyr::summarize_each(dplyr::funs(median, sd, se=sd(.)/sqrt(n())), cpm_expr) %>%
  dplyr::arrange(gene, Stage)

TEAD_expr_human_NAFLD <- df2 %>%
  dplyr::select(gene, Stage, median) %>%
  tidyr::pivot_wider(names_from = 'Stage', values_from = 'median')

p <- lapply(TEAD_genes, function(x){
```
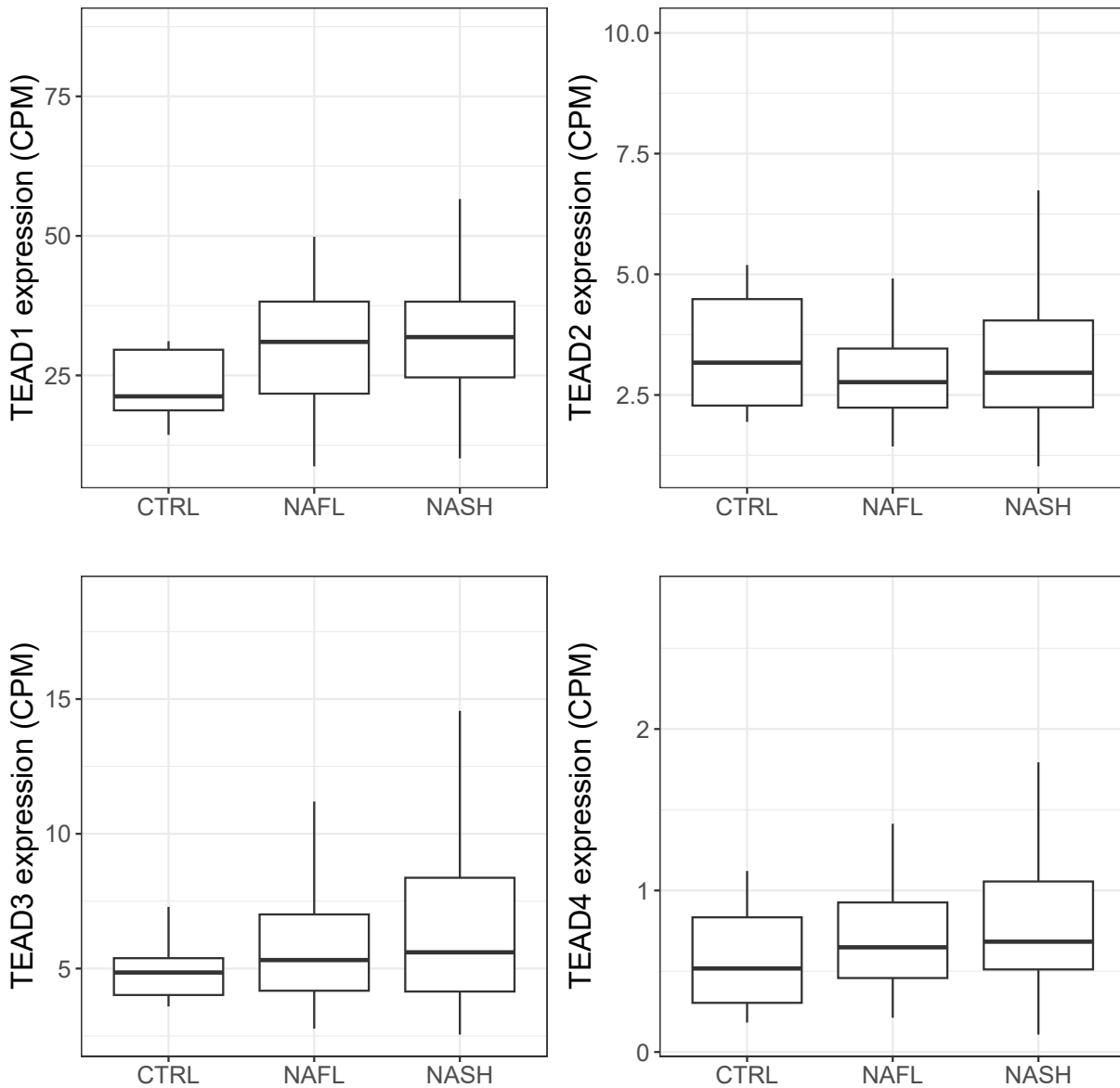
```
ggplot(df %>% dplyr::filter(gene == x) , aes(x=Stage, y=cpm_expr)) +
geom_boxplot(outlier.shape = NA) +
coord_cartesian(ylim = quantile(df %>% dplyr::filter(gene == x) %>% pull(cpm_expr), c(0, 0.999))) +
xlab("") +
ylab(paste(x,"expression (CPM)")) +
theme_bw() +
theme(text = element_text(size=15)) +
theme(legend.position="none")
})

patchwork::wrap_plots(p, nrow=2, ncol=2, byrow=T)
```



## Mouse single-cell

```
TEAD_genes <- c('Tead1','Tead2','Tead3','Tead4')

set.seed(22)
dat <- liver$mouseStSt$All
```

```r
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(sex == 'male' & annot != 'HsPCs')
dat <- subset(dat, cells = subs$cellid)

DefaultAssay(dat) <- "RNA"
Idents(dat) <- dat$annot

df <- AverageExpression(dat, slot = 'data', features = TEAD_genes) %>%
  as.data.frame() %>%
  tibble::rownames_to_column('gene') %>%
  tidyr::pivot_longer(colnames(.)[-c(1)], names_to = 'cell_type', values_to = 'expr') %>%
  dplyr::mutate(cell_type = gsub('RNA\\.', '', cell_type)) %>%
  dplyr::mutate(cell_type = gsub('\\.', ' ', cell_type)) %>%
  dplyr::mutate(cell_type = gsub('  ', '\\. ', cell_type)) %>%
  dplyr::mutate(cell_type = gsub('Monocytes\\.  Monocyte ', 'Monocytes & Monocyte-', cell_type)) %>%
  dplyr::mutate(cell_type = replace(cell_type, cell_type == 'Fibroblasts', 'Stromal cells')) %>%
  dplyr::mutate(cell_type = factor(cell_type, levels = names(colPals$celltypes)))

TEAD_expr_mouse_single_cell_stst <- df %>%
  tidyr::pivot_wider(names_from = 'gene', values_from = 'expr') %>%
  dplyr::arrange(factor(cell_type, levels = names(colPals$celltypes)))

ggplot(df, aes(x=cell_type, y=expr, fill=cell_type)) +
  geom_col(position = position_dodge(width=0.9), color="black") +
  scale_fill_manual(values = alpha(colPals$celltypes, 0.9)) +
  xlab("") +
  ylab("Normalized expression") +
  facet_wrap(~gene, scales = 'free_y') +
  theme_bw() +
  theme(axis.text.x =element_text(angle=90, hjust=0.95, vjust=0.5),
        text = element_text(size=12)) +
  theme(legend.position="none")
```
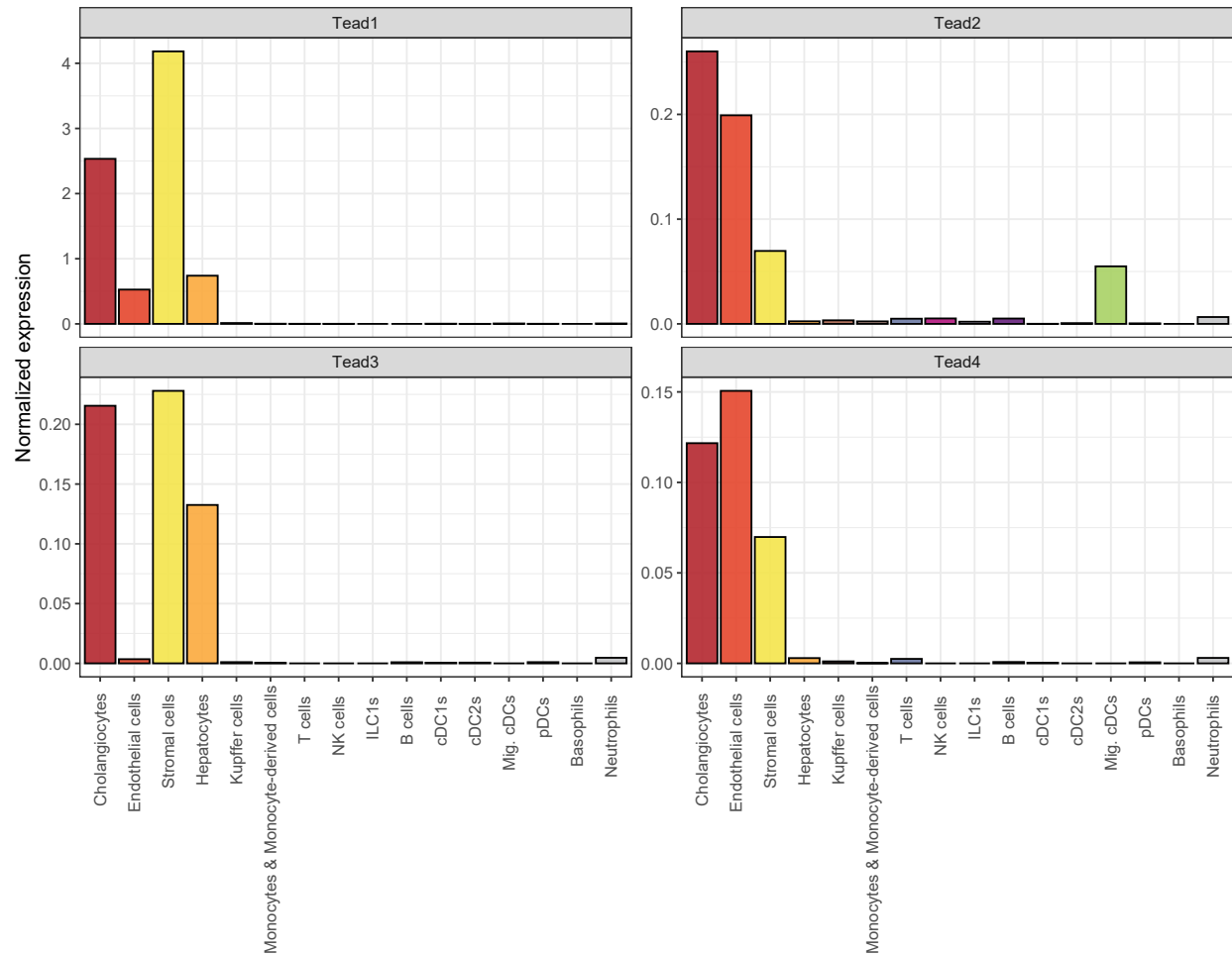
# PHH spheroids (control + TEAD inhibitors)

```r
TEAD_genes <- c('TEAD1','TEAD2','TEAD3','TEAD4')

df <- PHH_RNAseq_tpm_mean %>%
  dplyr::filter(external_gene_name %in% TEAD_genes) %>%
  dplyr::select(external_gene_name, control, TEADsf, TEADap) %>%
  dplyr::rename(gene = external_gene_name) %>%
  dplyr::mutate(gene = factor(gene, levels = TEAD_genes)) %>%
  dplyr::arrange(gene) %>%
  pivot_longer(control:TEADap, names_to = 'condition', values_to = 'tpm_expr')

TEAD_expr_PHH_spheroids <- df %>%
  tidyr::pivot_wider(names_from = 'condition', values_from = 'tpm_expr')

ggplot(df, aes(x=condition, y=tpm_expr)) +
  geom_col(position = position_dodge(width=0.9), color="black") +
  scale_fill_manual(values = alpha(colPals$celltypes, 0.9)) +
  xlab("") +
  ylab("TPM expression") +
  facet_wrap(~gene, scales = 'free_y') +
  theme_bw() +
  theme(text = element_text(size=12)) +
  theme(legend.position="none")
```

6
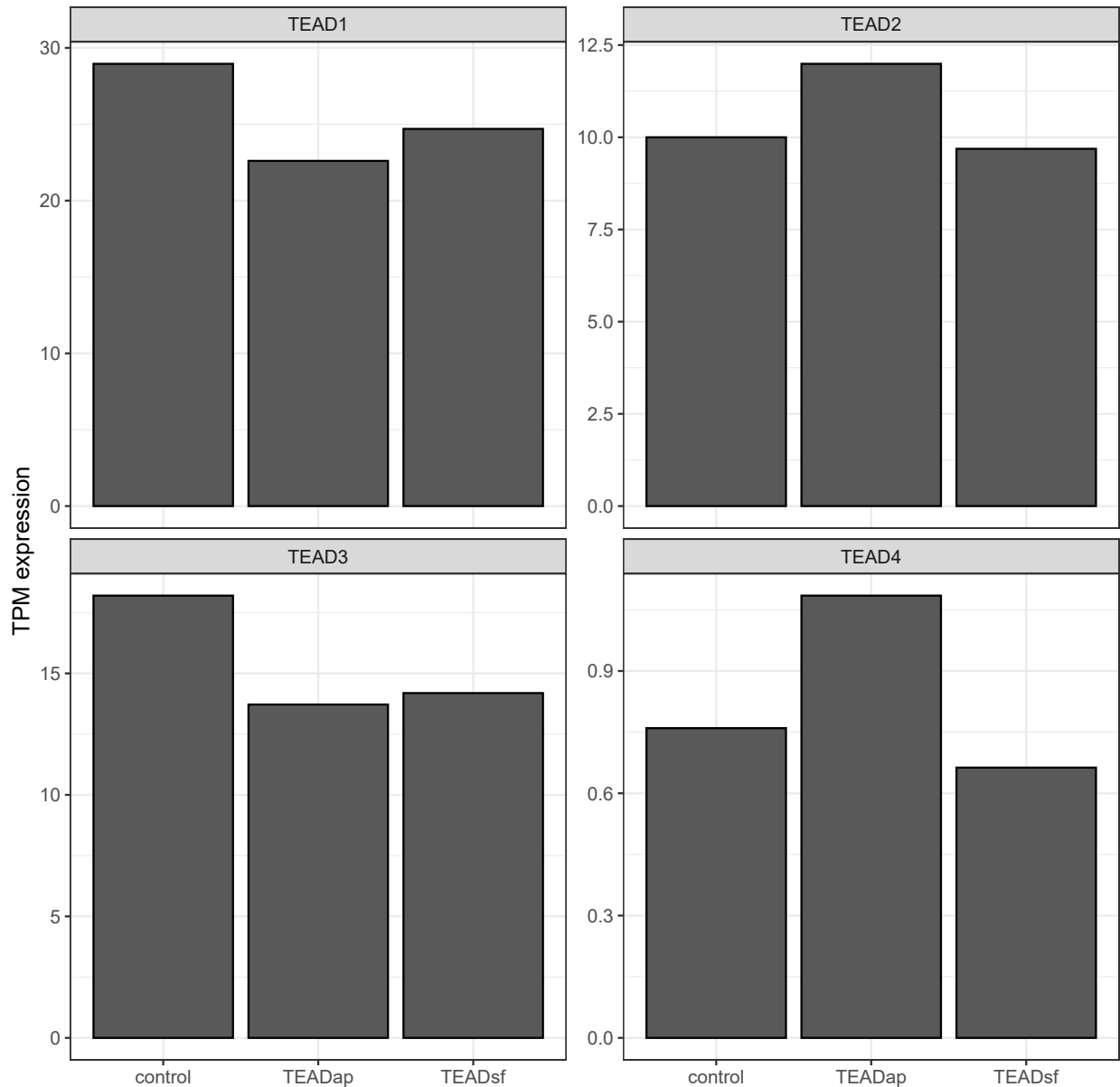
## ER expression

### Mouse (CD, HFD & HFD + ER treatments)

```r
ER_genes <- c('Esr1','Esr2')

df <- mouse_RNAseq$tpm %>%
  tibble::rownames_to_column(var = 'gene') %>%
  dplyr::mutate(gene = dplyr::recode(gene,
                                     !!!setNames(mouse_RNAseq$gene_ann$external_gene_name,
                                                 mouse_RNAseq$gene_ann$geneID))) %>%
  dplyr::filter(gene %in% ER_genes) %>%
  dplyr::mutate(gene = factor(gene, levels = ER_genes)) %>%
  dplyr::arrange(gene) %>%
  tidyr::pivot_longer(CD_female_1:PPT_HFD_male_3, names_to = 'sample', values_to = 'tpm_expr') %>%
  dplyr::left_join(mouse_RNAseq$design_meta, by = 'sample') %>%
  dplyr::filter(!condition %in% c('CDf','HFDf')) %>%
```

```
    dplyr::mutate(condition = factor(condition, levels = names(colPals$conditions)[-c(1,2)]))

df2 <- df %>%
  dplyr::group_by(gene, condition) %>%
  dplyr::select(gene, condition, tpm_expr) %>%
  dplyr::summarize_each(dplyr::funs(mean, sd, se=sd(.)/sqrt(n())), tpm_expr) %>%
  dplyr::arrange(gene, condition)

ER_expr_mouse <- df2 %>%
  dplyr::select(gene, condition, mean) %>%
  tidyr::pivot_wider(names_from = 'condition', values_from = 'mean')

p <- lapply(ER_genes, function(x){
  ggplot() +
  geom_errorbar(data=df2 %>% dplyr::filter(gene == x), aes(x=condition, ymin=mean-sd, ymax=mean+sd),
                position= position_dodge(width=0.9), width=0.3) +
  geom_col(data=df2 %>% dplyr::filter(gene == x), aes(x=condition, y=mean, fill=condition),
           position = position_dodge(width=0.9), color="black") +
  geom_point(data=df %>% dplyr::filter(gene == x), aes(x=condition, y=tpm_expr),
             shape=21, size=2.5, alpha=0.5, fill="darkgrey", position = position_dodge(width=0.9)) +
  scale_fill_manual(values = alpha(colPals$conditions, 0.9)) +
  xlab("") +
  ylab(paste(x,"expression (TPM)")) +
  theme_bw() +
  theme(axis.text.x =element_text(angle=90, hjust=0.95, vjust=0.5),
        text = element_text(size=15)) +
  theme(legend.position="none")
})

patchwork::wrap_plots(p, nrow=1, ncol=2, byrow=T)
```
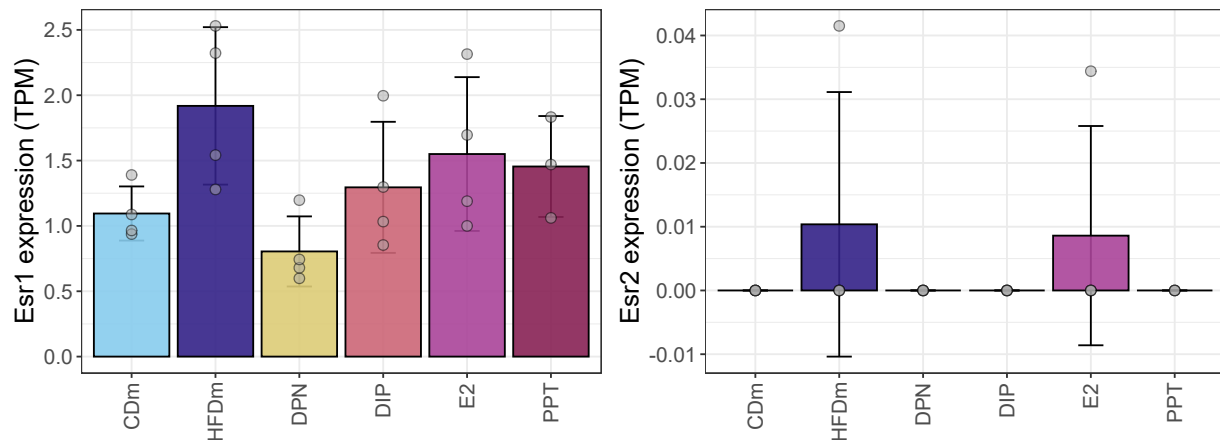


## Human NAFLD patient cohort

```
ER_genes <- c('ESR1','ESR2')

df <- cohort_data$Govaere$cpm_filt %>%
  tibble::rownames_to_column(var = 'gene') %>%
  dplyr::filter(gene %in% ER_genes) %>%
  dplyr::mutate(gene = factor(gene, levels = ER_genes)) %>%
  dplyr::arrange(gene) %>%
  tidyr::pivot_longer(cohort_data$Govaere$meta$Patient, names_to = 'Patient', values_to = 'cpm_expr') %>%
  dplyr::left_join(cohort_data$Govaere$meta, by = 'Patient') %>%
  dplyr::mutate(Stage = factor(Stage, levels = c('CTRL','NAFL','NASH')))

df2 <- df %>%
  dplyr::group_by(gene, Stage) %>%
  dplyr::select(gene, Stage, cpm_expr) %>%
  dplyr::summarize_each(dplyr::funs(median, sd, se=sd(.)/sqrt(n())), cpm_expr) %>%
  dplyr::arrange(gene, Stage)

ER_expr_human_NAFLD <- df2 %>%
  dplyr::select(gene, Stage, median) %>%
  tidyr::pivot_wider(names_from = 'Stage', values_from = 'median')
```
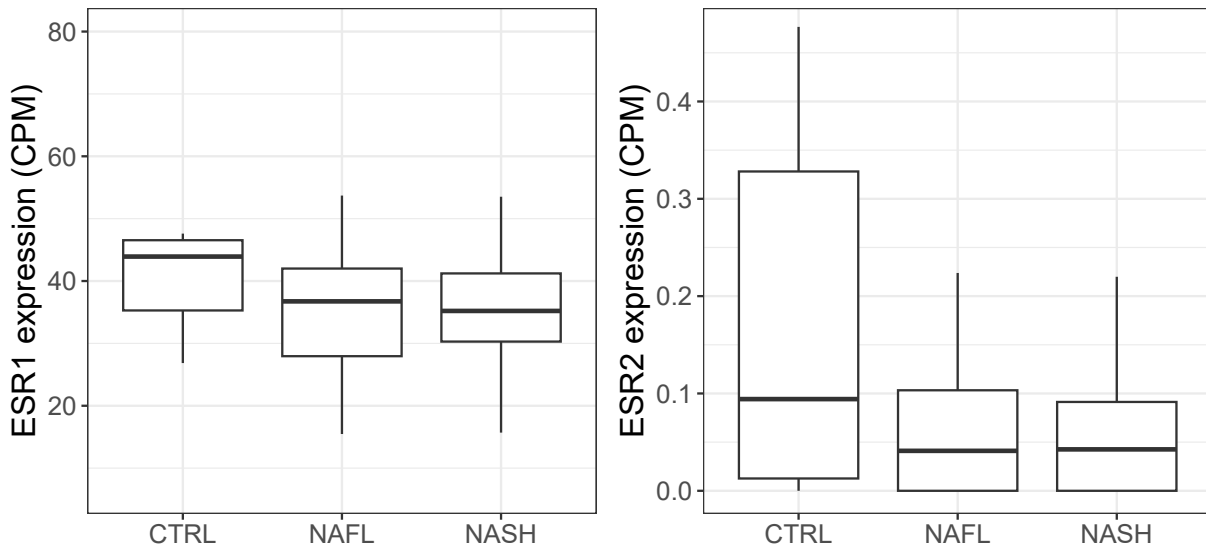
```
p <- lapply(ER_genes, function(x){
  ggplot(df %>% dplyr::filter(gene == x) , aes(x=Stage, y=cpm_expr)) +
  geom_boxplot(outlier.shape = NA) +
  coord_cartesian(ylim = quantile(df %>% dplyr::filter(gene == x) %>% pull(cpm_expr), c(0, 0.999))) +
  xlab("") +
  ylab(paste(x,"expression (CPM)")) +
  theme_bw() +
  theme(text = element_text(size=15)) +
  theme(legend.position="none")
})

patchwork::wrap_plots(p, nrow=1, ncol=2, byrow=T)
```



## Mouse single-cell

```
ER_genes <- c('Esr1','Esr2')

set.seed(22)
dat <- liver$mouseStSt$All
subs <- dat@meta.data %>%
  tibble::rownames_to_column(var = 'cellid') %>%
  dplyr::left_join(liver_meta, by = 'sample') %>%
  dplyr::filter(sex == 'male' & annot != 'HsPCs')
dat <- subset(dat, cells = subs$cellid)

DefaultAssay(dat) <- "RNA"
Idents(dat) <- dat$annot

df <- AverageExpression(dat, slot = 'data', features = ER_genes) %>%
  as.data.frame() %>%
  tibble::rownames_to_column('gene') %>%
  tidyr::pivot_longer(colnames(.)[-c(1)], names_to = 'cell_type', values_to = 'expr') %>%
  dplyr::mutate(cell_type = gsub('RNA\\.', '', cell_type)) %>%
  dplyr::mutate(cell_type = gsub('\\.', ' ', cell_type)) %>%
  dplyr::mutate(cell_type = gsub('  ', '\\. ', cell_type)) %>%
  dplyr::mutate(cell_type = gsub('Monocytes\\.  Monocyte ', 'Monocytes & Monocyte-', cell_type)) %>%
  dplyr::mutate(cell_type = replace(cell_type, cell_type == 'Fibroblasts', 'Stromal cells')) %>%
  dplyr::mutate(cell_type = factor(cell_type, levels = names(colPals$celltypes)))

ER_expr_mouse_single_cell_stst <- df %>%
  tidyr::pivot_wider(names_from = 'gene', values_from = 'expr') %>%
  dplyr::arrange(factor(cell_type, levels = names(colPals$celltypes)))

ggplot(df, aes(x=cell_type, y=expr, fill=cell_type)) +
  geom_col(position = position_dodge(width=0.9), color="black") +
  scale_fill_manual(values = alpha(colPals$celltypes, 0.9)) +
  xlab("") +
```
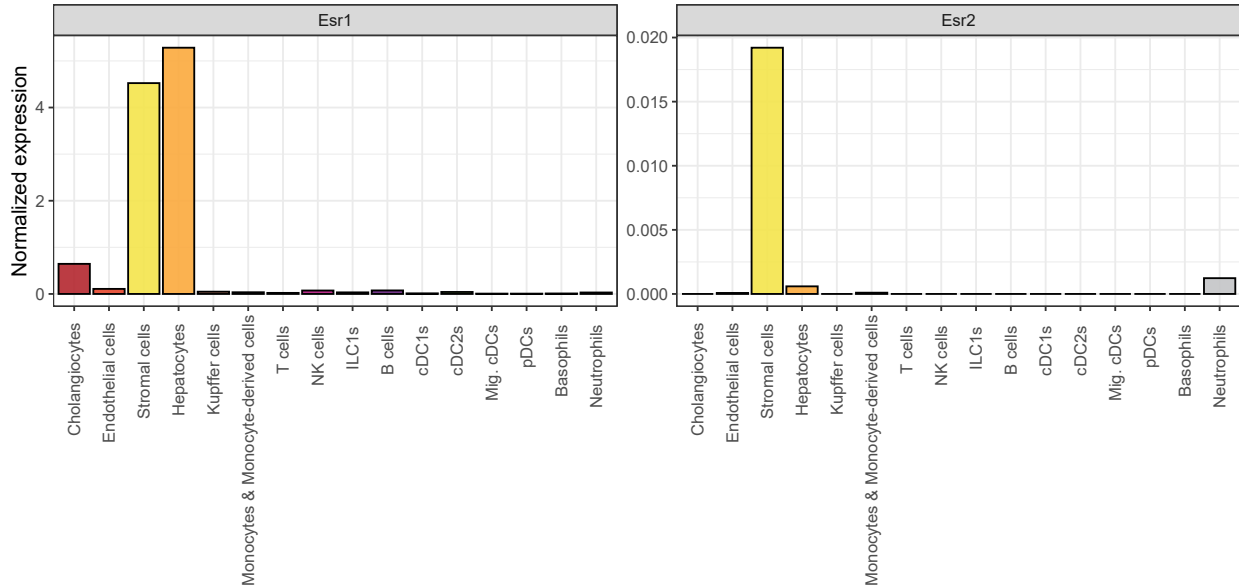
```
    ylab("Normalized expression") +
    facet_wrap(~gene, scales = 'free_y') +
    theme_bw() +
    theme(axis.text.x =element_text(angle=90, hjust=0.95, vjust=0.5),
          text = element_text(size=12)) +
    theme(legend.position="none")
```



## PHH spheroids (control + TEAD inhibitors)

```
ER_genes <- c('ESR1','ESR2')

df <- PHH_RNAseq_tpm_mean %>%
  dplyr::filter(external_gene_name %in% ER_genes) %>%
  dplyr::select(external_gene_name, control, TEADsf, TEADap) %>%
  dplyr::rename(gene = external_gene_name) %>%
  dplyr::mutate(gene = factor(gene, levels = ER_genes)) %>%
  dplyr::arrange(gene) %>%
  pivot_longer(control:TEADap, names_to = 'condition', values_to = 'tpm_expr')

ER_expr_PHH_spheroids <- df %>%
  tidyr::pivot_wider(names_from = 'condition', values_from = 'tpm_expr')

ggplot(df, aes(x=condition, y=tpm_expr)) +
  geom_col(position = position_dodge(width=0.9), color="black") +
  scale_fill_manual(values = alpha(colPals$celltypes, 0.9)) +
  xlab("") +
  ylab("TPM expression") +
  facet_wrap(~gene, scales = 'free_y') +
  theme_bw() +
  theme(text = element_text(size=12)) +
  theme(legend.position="none")
```
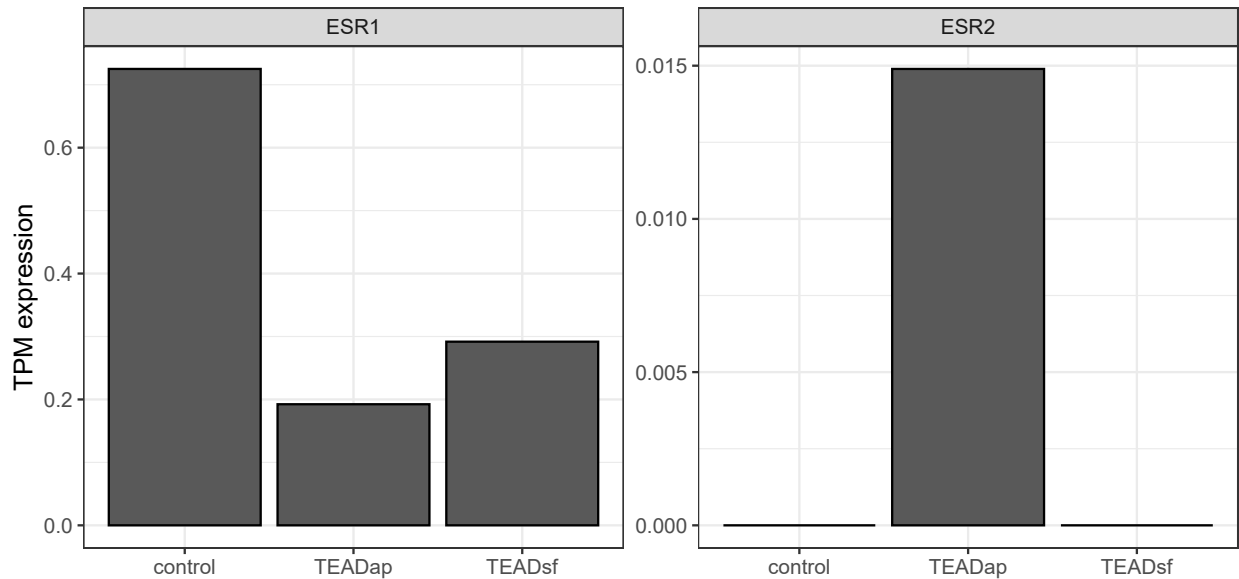
## Exports

```r
TEAD_expr <- list(mouse_HFD_ER_agonists = TEAD_expr_mouse,
                  mouse_liver_cell_types = TEAD_expr_mouse_single_cell_stst,
                  human_NAFLD_patients = TEAD_expr_human_NAFLD,
                  PHH_TEAD_inhibitors = TEAD_expr_PHH_spheroids)
saveRDS(TEAD_expr, file = 'results/TEAD_family_expression.rds')

ER_expr <- list(mouse_HFD_ER_agonists = ER_expr_mouse,
                mouse_liver_cell_types = ER_expr_mouse_single_cell_stst,
                human_NAFLD_patients = ER_expr_human_NAFLD,
                PHH_TEAD_inhibitors = ER_expr_PHH_spheroids)
saveRDS(ER_expr, file = 'results/ER_family_expression.rds')
```

## SessionInfo

```r
sessionInfo()
```

```
## R version 4.2.1 (2022-06-23 ucrt)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19045)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.utf8
## [2] LC_CTYPE=English_United States.utf8
## [3] LC_MONETARY=English_United States.utf8
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.utf8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] SeuratObject_4.1.3 Seurat_4.3.0.1     lubridate_1.9.2    forcats_1.0.0
##  [5] stringr_1.5.0      dplyr_1.1.0        purrr_1.0.1        readr_2.1.4
##  [9] tidyr_1.3.0        tibble_3.2.1       ggplot2_3.4.2      tidyverse_2.0.0
##
## loaded via a namespace (and not attached):
##   [1] Rtsne_0.16             colorspace_2.0-3       deldir_1.0-9
##   [4] ellipsis_0.3.2         ggridges_0.5.4         spatstat.data_3.0-1
##   [7] rstudioapi_0.15.0      farver_2.1.1           leiden_0.4.3
##  [10] listenv_0.9.0          ggrepel_0.9.2          fansi_1.0.3
```

```
##   [13] codetools_0.2-19       splines_4.2.1        knitr_1.43
##   [16] polyclip_1.10-4        jsonlite_1.7.2       ica_1.0-3
##   [19] cluster_2.1.4          png_0.1-8            uwot_0.1.16
##   [22] spatstat.sparse_3.0-2  shiny_1.7.4.1        sctransform_0.3.5
##   [25] compiler_4.2.1         httr_1.4.6           Matrix_1.5-3
##   [28] fastmap_1.1.0          lazyeval_0.2.2       cli_3.4.1
##   [31] later_1.3.1            htmltools_0.5.5      tools_4.2.1
##   [34] igraph_1.3.5           gtable_0.3.3         glue_1.6.2
##   [37] RANN_2.6.1             reshape2_1.4.4       Rcpp_1.0.9
##   [40] scattermore_1.2        vctrs_0.6.2          nlme_3.1-162
##   [43] spatstat.explore_3.2-1 progressr_0.13.0     lmtest_0.9-40
##   [46] spatstat.random_3.1-5  xfun_0.39            globals_0.16.2
##   [49] timechange_0.2.0       mime_0.12            miniUI_0.1.1.1
##   [52] lifecycle_1.0.3        irlba_2.3.5.1        goftest_1.2-3
##   [55] future_1.33.0          MASS_7.3-60          zoo_1.8-12
##   [58] scales_1.2.1           hms_1.1.3            promises_1.2.0.1
##   [61] spatstat.utils_3.0-3   parallel_4.2.1       RColorBrewer_1.1-3
##   [64] yaml_2.3.7             reticulate_1.18      pbapply_1.7-2
##   [67] gridExtra_2.3          stringi_1.7.8        highr_0.10
##   [70] rlang_1.1.1            pkgconfig_2.0.3      matrixStats_0.63.0
##   [73] evaluate_0.21          lattice_0.20-41      tensor_1.5
##   [76] ROCR_1.0-11            labeling_0.4.2       patchwork_1.1.2
##   [79] htmlwidgets_1.6.2      cowplot_1.1.1        tidyselect_1.2.0
##   [82] parallelly_1.36.0      RcppAnnoy_0.0.21     plyr_1.8.8
##   [85] magrittr_2.0.3         R6_2.5.1             generics_0.1.3
##   [88] pillar_1.9.0           withr_2.5.0          fitdistrplus_1.1-11
##   [91] abind_1.4-5            survival_3.5-5       sp_2.0-0
##   [94] future.apply_1.11.0    KernSmooth_2.23-22   utf8_1.2.2
##   [97] spatstat.geom_3.2-2    plotly_4.10.2        tzdb_0.4.0
##  [100] rmarkdown_2.23         grid_4.2.1           data.table_1.14.6
##  [103] digest_0.6.30          xtable_1.8-4         httpuv_1.6.11
##  [106] munsell_0.5.0          viridisLite_0.4.2
```